

First record of *Zhangixalus franki* Ninh, Nguyen, Orlov, Nguyen & Ziegler, 2020 (Anura, Rhacophoridae) from China

Shuo Liu¹, Hong Hui², Dingqi Rao²

¹ Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan Province 650223, China

² Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan Province 650223, China

<http://zoobank.org/128ADF67-AC46-456F-ACD0-F00AB7FF4ECB>

Corresponding author: Dingqi Rao (raodq@mail.kiz.ac.cn)

Academic editor: Peter Mikulíček ♦ Received 29 September 2020 ♦ Accepted 21 October 2020 ♦ Published 2 November 2020

Abstract

We record *Zhangixalus franki* Ninh, Nguyen, Orlov, Nguyen & Ziegler, 2020 for the first time from China based on two specimens collected from Laoshan Nature Reserve, Yunnan Province. Morphologically, the newly collected specimens from China mostly agree with the type series from Vietnam. Phylogenetically, these individuals were placed in a clade with *Z. franki* from Vietnam based on DNA sequences of the mitochondrial 16S rRNA gene. Our finding brings the species number of the genus *Zhangixalus* to 29 in China.

Key Words

distribution, mtDNA, new record, phylogeny, tree frog, Yunnan

The genus *Zhangixalus* Li, Jiang, Ren & Jiang, 2019 was split from *Rhacophorus* Kuhl & van Hasselt, 1822 by Jiang et al. (2019) and *Polypedates dugritei* David, 1872 was designated as the type species. This genus is characterized by body size relatively large (SVL 30–120 mm, above 50 mm in most species); snout rounded; snout, upper eyelid and tarsal projections absent; dermal folds along forearm or tarsus absent; terminal phalanges of finger and toes Y-shaped; skin of dorsal surfaces smooth, or scattered with small tubercles; webbed fingers; dorsal coloration green in most species; and iris without “X” shaped pattern (Jiang et al. 2019). During the breeding period the breeding pairs produce white foam nests (Jiang et al. 2019). *Zhangixalus* currently contains 39 species with a distribution range from northeastern India, Bangladesh, Nepal, Bhutan, southern China, southern Japan, Myanmar, Indochina, and northern Thailand (Frost 2020; Jiang et al. 2019). Of the 39 recognized species, 28 species are distributed in China (Yu et al. 2019; AmphibiaChina 2020).

Zhangixalus franki is a species which was recently described from Ha Giang Province, northeast Vietnam (Ninh et al. 2020), and was known only from the type locality.

During our fieldwork in Laoshan Nature Reserve, Wenshan Prefecture, Yunnan Province, China from 2006 to 2012, we collected various specimens of tree frogs. After recent careful examination, we found two of them should be assigned to the recently named species *Z. franki* from comparisons in appearance and molecular analysis.

Specimens were fixed in 75% ethanol for storage after we had taken photographs and the specimens euthanized. Tissue samples of liver were preserved in 99% ethanol for molecular analysis. Specimens were deposited in Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ).

Total genomic DNA was extracted from liver tissues. A fragment encoding mitochondrial 16S rRNA gene was amplified and sequenced for two specimens collected from Yunnan, China. We used the primers 16Sar: 5'-CG-CCTGTTTACCAAAAACAT-3' and 16Sbr: 5'-CCG-

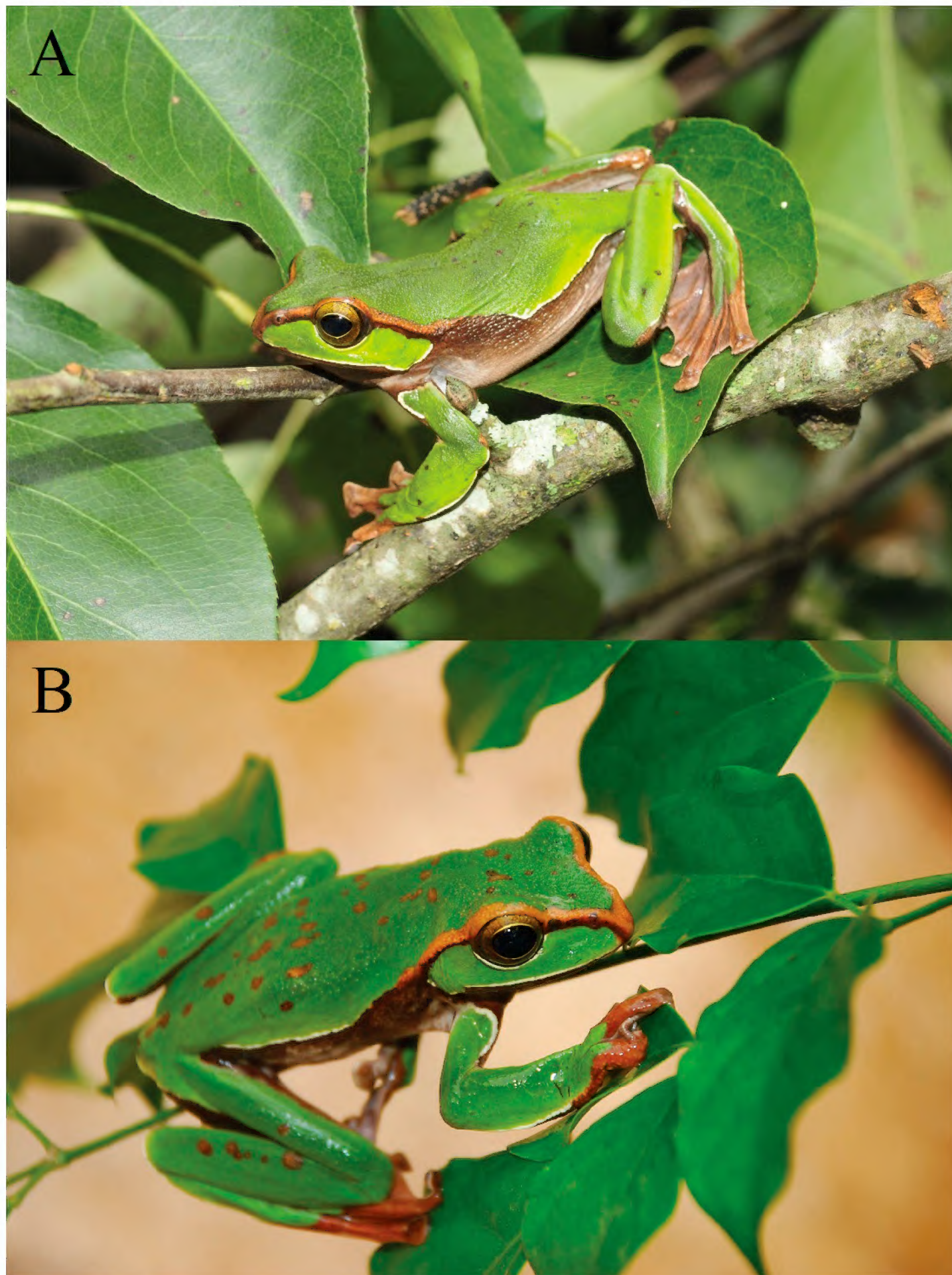


Figure 1. *Zhangixalus franki* from China in life. **A.** Adult male, voucher: KIZ 0654; **B.** Adult male, voucher: KIZ 1263.

GTTTGAAGCTCAGATCACGT-3' (Palumbi et al. 1991) to amplify the fragment. Sequences of 22 species of *Zhangixalus* and three species of *Rhacophorus* were used in combination with a sequence of *Buergeria buergeri* (Temminck & Schlegel, 1838) as an outgroup according to Ninh et al. (2020) and Nguyen et al. (2020). Homologous sequences were obtained from GenBank (Suppl. material 1: Table S1).

Sequences were aligned using MAFFT version 7 (Kato and Standley 2013) with the default parameters. Uncorrected pairwise distances between species were calculated in MEGA7 (Kumar et al. 2016). The substitution model selected GTR+G according to Ninh et al. (2020). Bayesian inference (BI) was performed in MrBayes 3.2.6

(Ronquist et al. 2012). Two runs were performed simultaneously with four Markov chains starting from a random tree. The chains were run for 1,000,000 generations and sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in after the standard deviation of split frequencies of the two runs was less than a value of 0.01. Afterwards, the remaining trees were used to create a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPPs). Maximum likelihood (ML) analysis was conducted in RaxmlGUI 1.5 (Silvestro and Michalak 2012) with 1000 rapid bootstrap replicates.

Two collected specimens of *Z. franki* were adult males found in Laoshan Nature Reserve, Xiajinchang Township,

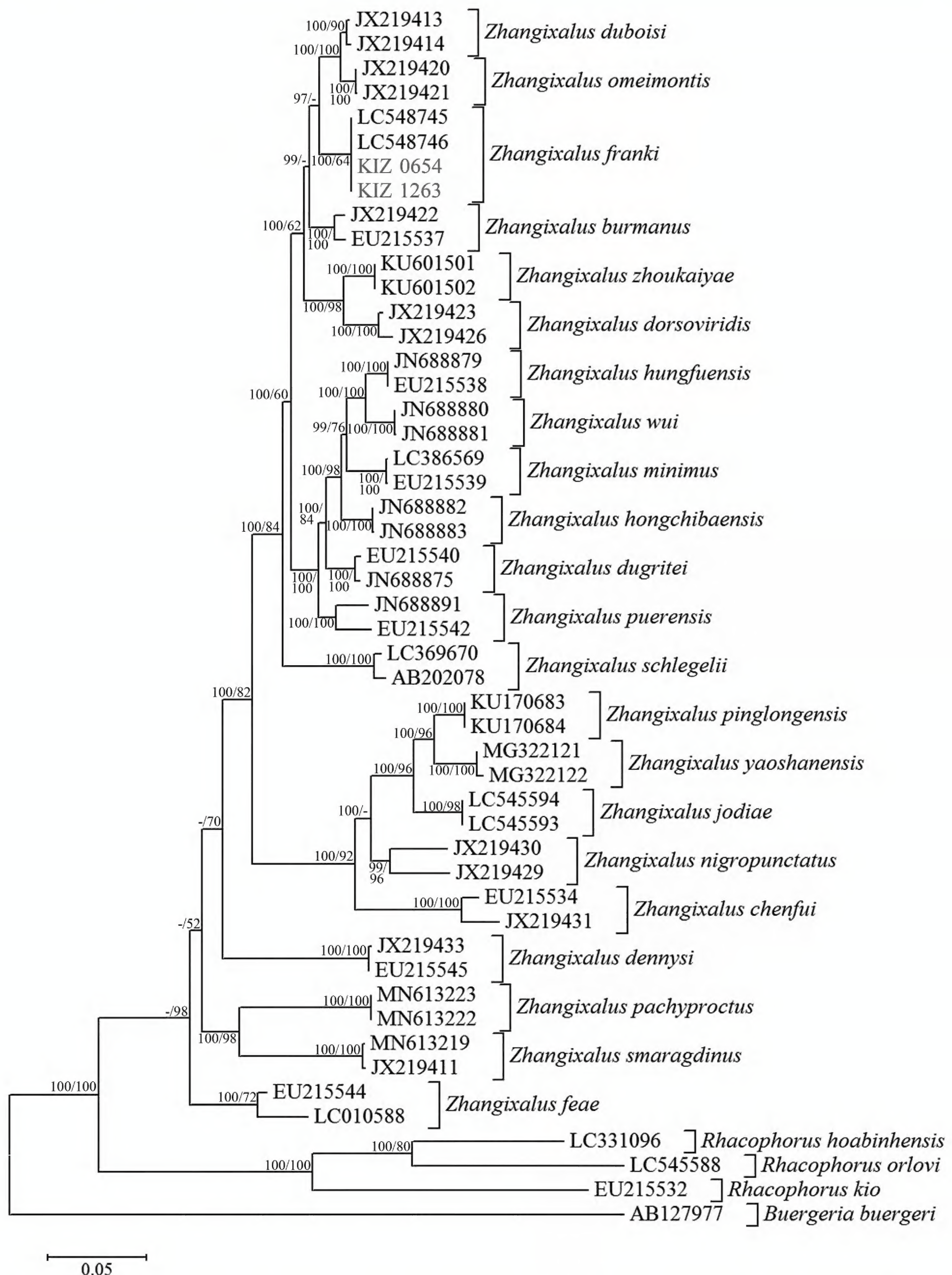


Figure 2. Phylogenetic relationships (Bayesian inference and Maximum likelihood) of the genus *Zhangixalus* inferred from 16S rRNA gene. Numbers before slashes indicate Bayesian posterior probabilities (only values above 70% are shown) and numbers after slashes indicate bootstrap support for Maximum likelihood analysis (only values above 50% are shown). The specimens collected in China are indicated by red.

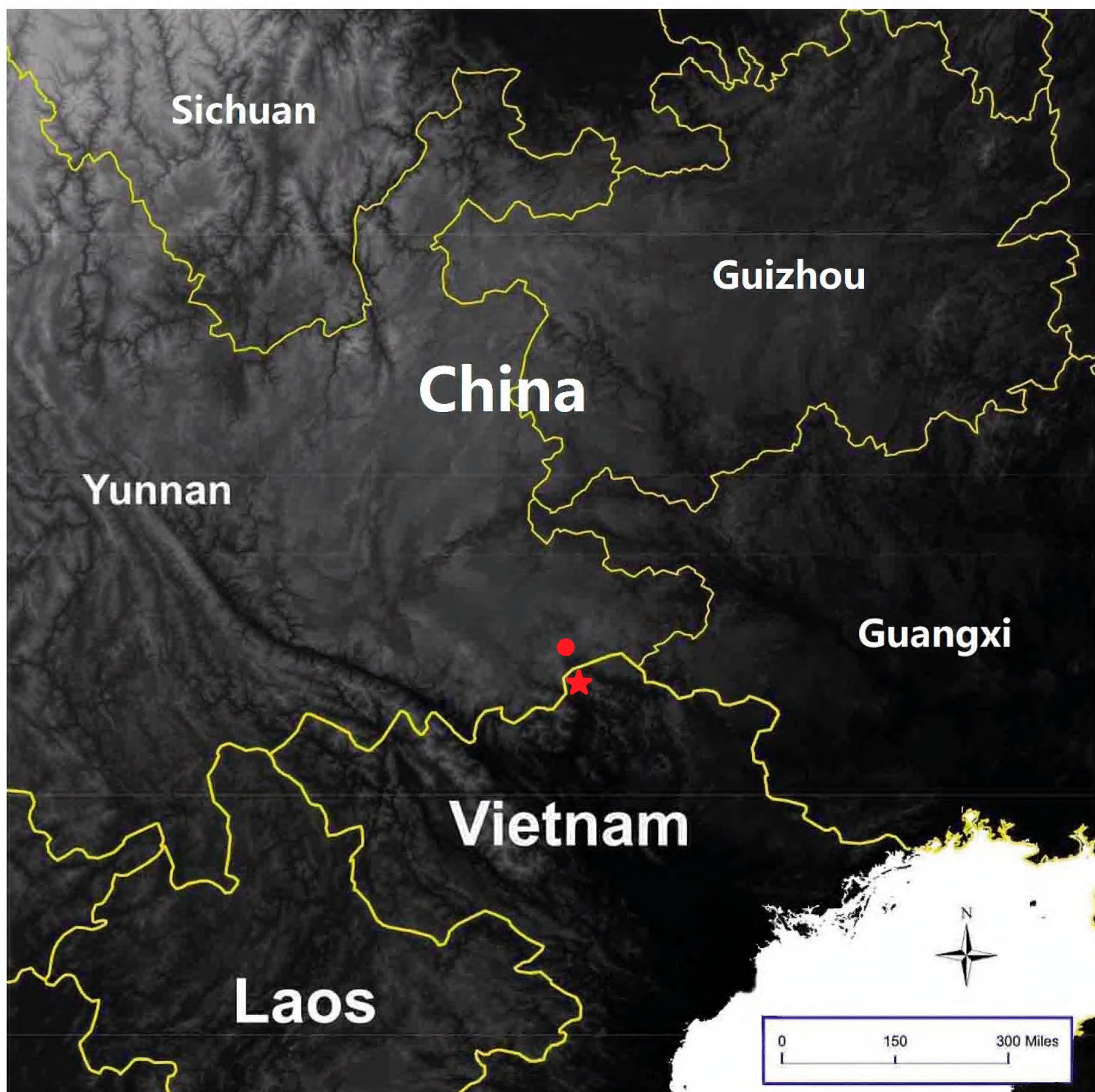


Figure 3. Map showing the collection site of *Zhangixalus franki* from Yunnan Province, China (red dot) and the type locality of *Z. franki* from Ha Giang Province, Vietnam (red pentacle).

Malipo County, Wenshan Autonomous Prefecture, Yunnan Province, China. A male KIZ 0654 collected on 20 June 2006 (23°10'6"N, 104°48'52"E, 1680 m elevation), a male KIZ 1263 collected on 8 July 2012 (23°11'22"N, 104°48'31"E, 1350 m elevation).

The two specimens have a similar appearance: dorsal surface of head and body green with some small brown spots, various in size and shape; tympanum region green; a brown stripe running from tip of snout, along canthus rostralis, bordering upper eyelid, along supratympanic fold to posterior of axilla; dorsal surface of fore and hind limbs green, fingers and toes brown; upper part of flank dark brown, axilla, lower part of flank and belly grey; white stripe present along edge of upper jaw, limb insertions, and dermal fringes of fore and hind limbs; flank

with white stripe, separating upper green part from lower dark brown part; ventral surface of body and limbs grey; iris bronze, pupil black; dorsal surface of head and body smooth with a lot of very small tubercles, canthus rostralis smooth, supratympanic folds smooth with many very small tubercles, throat and chest smooth, belly rough, ventral surface of fore and hind limbs smooth (Fig. 1). These characters mostly agree with the type series of *Z. franki* apart from a few differences (fingers, toes and flanks of the two specimens from China are brown while they are grey in the original description; dorsal surface of head and body with a lot of very small tubercles while they are smooth in the original description).

The sequence alignment for a fragment encoding 16S rRNA gene was 510 bp in length. The sequences of the

two specimens from China were nested in the genus *Zhangixalus* and were clustered together with the two specimens of *Z. franki* from Vietnam (Fig. 2). There was no genetic divergence between the four individuals of *Z. franki* (Suppl. material 2: Table S2).

The two specimens of *Z. franki* from Yunnan, China were collected before this species was described and named. These two specimens were ignored all the time until we carefully reexamined the collected material, and found they belonged to the species *Z. franki*. We provide evidence for the first time that this species is also distributed in China. Our record increases the total number of *Zhangixalus* species in China to 29.

In Yunnan, specimens of *Z. franki* were found on trees at night, close to small ponds in undisturbed evergreen forest. No females and eggs were found. Geographically, this new location in Yunnan is about 10 km away from the type locality of the species in Vietnam. This species probably also occurs in other parts of Wenshan Autonomous Prefecture, Yunnan Province, China (Fig. 3).

Acknowledgements

We would like to thank Decai Ouyang and Jiazhong Wang for assistance in the field. Thanks to Wenjing Jiang for providing photos. Thanks also to our colleagues for their help and advice. We also thank the reviewers for their valuable comments on the manuscript.

References

AmphibiaChina (2020) The database of Chinese amphibians. Electronic Database. <http://www.amphibiachina.org> [Accessed on 20 September 2020]

Frost DR (2020) Amphibian Species of the World: an Online Reference. Version 6.1. Electronic Database. <https://amphibiansoftheworld.amnh.org/index.php> [Accessed on 20 September 2020]

Jiang DC, Jiang K, Ren JL, Wu J, Li JT (2019) Resurrection of the genus *Leptomantis*, with description of a new genus to the family Rhacophoridae (Amphibia: Anura). Asian Herpetological Research 10: 1–12.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. <https://doi.org/10.1093/molbev/mst010>

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>

Nguyen TT, Ninh HT, Orlov N, Nguyen TQ, Ziegler T (2020) A new species of the genus *Zhangixalus* (Amphibia: Rhacophoridae) from Vietnam. Journal of Natural History 54: 257–273. <https://doi.org/10.1080/00222933.2020.1754484>

Ninh HT, Nguyen TT, Orlov N, Nguyen TQ, Ziegler T (2020) A New Species of the Genus *Zhangixalus* (Amphibia: Rhacophoridae) from

Vietnam. European Journal of Taxonomy 688: 1–18. <https://doi.org/10.5852/ejt.2020.688>

Palumbi SR, Martin A, Romano S, McMillan W, Stice L, Grabowski G (1991) The Simple Fool’s Guide to PCR. University of Hawaii Press, Honolulu, 94 pp.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12(4): 335–337. <https://doi.org/10.1007/s13127-011-0056-0>

Yu GH, Hui H, Hou M, Wu ZJ, Rao DQ, Yang JX (2019) A new species of *Zhangixalus* (Anura: Rhacophoridae), previously confused with *Zhangixalus smaragdinus* (Blyth, 1852). Zootaxa 4711: 275–292. <https://doi.org/10.11646/zootaxa.4711.2.3>

Supplementary material 1

Table S1. Samples used for DNA analysis in this study

Authors: Shuo Liu, Hong Hui, Dingqi Rao
Data type: DNA samples
Explanation note: Samples used for DNA analysis in this study.
Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: <https://doi.org/10.3897/herpetozoa.32.e59114.suppl1>

Supplementary material 2

Table S2. Pairwise distances (%) between and within homologous species and *Zhangixalus franki*

Authors: Shuo Liu, Hong Hui, Dingqi Rao
Data type: Pairwise distances
Explanation note: Pairwise distances (%) between and within homologous species and *Zhangixalus franki*.
Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: <https://doi.org/10.3897/herpetozoa.32.e59114.suppl2>